December 11, 1957.

The Editors
The Journal of Biological Chemistry
Yale University
New Haven, Connecticut

Gentlemen:

I have examined with considerable care your letter of December 5 regarding the two papers on the Enzymatic Synthesis of DNA, submitted by my colleagues and myself. As always, I am grateful for suggestions and criticisms and, as you will note, have incorporated these in the manuscript. There are, however, several points raised in your letter which in my judgment are not well founded and if accepted would damage the presentation. I have made a copy of your letter for your use with this letter in order to facilitate reference by numbers to the points discussed.below:

1. You require that, in order to qualify for the designation of DNA, our product be a "polymer of very high molecular weight and viscosity" (your letter, page 2). You have apparently overlooked our statement on page 13, line 5, paper II, that "the sedimentation characteristics of the DNA produced were found in preliminary studies to be similar to those of the calf thymus DNA". We had also demonstrated by ultracentrifugal and viscometric studies that the enzymatically synthesized product has all the characteristics of the long, rigid molecules in standard preparations of DNA isolated from natural sources. This additional information has now been added to this section of the discussion. These studies will be reported in detail in the near future. I firmly believe that within the limits of current scientific usage, we are justified in considering the product of our enzymatic reaction to be DNA.

2. In your pencilled comment on page 2, paper I, you seem to want to reserve the term DNA for material of proven biologic specificity. Obviously this would limit the use of the term to a few reports on "transforming factor", and the Editorial Board would then have to introduce a new term to cover the vast literature on DNA in which DNA is described and studied physically and chemically with no reference to biologic activity.

3. You question why T2 phage DNA has not been used as a "primer" in this investigation. You have failed to notice a statement regarding this on line 1, page 13, paper II. For your interest, we have carried out investigations on this point over the past year which are as yet incomplete, but have led to the tentative conclusion that DNA isolated from a variety of natural sources is indeed a primer in our enzyme system provided the DNA is highly polymerized. Our analyses of the base ratios of the synthetic product indicate that there is a relationship between the primer and the enzymatic product, but these results must be extended before they warrant publication. It would indeed be interesting to see what a nucleic acid not

containing deoxycytidylic acid might do, or plant DNA. A variety of interesting possibilities suggest themselves but these papers do not mark the end of our work on DNA synthesis, and we state on page 11, paper II, that "direct proof of the precise functions of DNA ..... is being sought." As for RNA, it is explicitly stated in our previous publication (see reference 7, paper I) that RNA does not act as a primer.

4. Regarding the description of the periodate treatment of ATP, we have intended this section to provide sufficient information to permit the reader to prepare dATP essentially free of ATP, and I believe this section is adequately described and documented for this purpose. It is not our intention here to study the detailed chemistry of this reaction.

5. You request condensation of the paragraph describing DNases. I believe you fail to appreciate the critical importance of recognizing the existence of several distinct DNases and of monitoring the removal of each of them in order to obtain net DNA synthesis, and to study the kinetics and details of the reaction mechanism.

6. You recommend omission of the paragraph on the preparation of P^{32}-labeled phage DNA. To my knowledge this is a currently useful description on how to prepare such material. We make no claim for its originality (references are given to the literature on which the procedure is based). However, people in this department and other institutions with good knowledge of phage techniques have found these details most helpful in getting good yields of this substance. If the Editors believe that this information is superfluous, despite this comment, I will agree to the omission of this section.

7. I regard this comment as insulting. It is unique in my experience as an editor and author. I can hardly believe that a member of the Editorial Board would agree to the communication of such insulting and destructive criticism.

8. You question the validity of regarding the incorporation of labeled inorganic pyrophosphate into deoxynucleoside triphosphates as a basis for reversal of the synthetic reaction. I would point out, as stated in the paper, that this reaction is completely dependent on the presence of high molecular-weight DNA, that is proceeds at a rate comparable to that of the synthetic rate, and that inorganic pyrophosphate inhibits the synthetic reaction to an extent predicted by its incorporation into the triphosphates. We have not said anywhere that small fragments can be pyrophosphorylyzed, but have speculated that the addition of single or short runs of nucleotides under conditions of "abortive" chain formation does lead to pyorphosphorylysis while polymerized DNA is pyrophosphorolyzed to only a limited extent. We agree that this section was not clearly stated; it has been restated and largely omitted. I would be eager to entertain plausible alternative interpretations.

9. The pencilled comment on page 2, Paper I, asks also "what properties are described in the previous paper"? These published papers have clearly stated that the product has chemical properties exhibited by native DNA under acid and alkaline conditions, has the same susceptibility to the action of pancreatic DNase, and that the sedimentation characteristics were not distinguishable from those isolated from calf thymus DNA (Fed. Proc., 16: 153 (1957); reference 7 in paper I). The study of the biologic activity of samples of DNA is a subject which is under investigation.

10. The enzyme activity has been purified over 4,000-fold. There has been no indication of separation of activities over this 4,000-fold range. It would be misleading to inject any indication that we suspect the existence of more than one enzyme at this point. I believe it is implicit in any intelligent understanding that no enzyme, no matter how many times recrystallized, is solely responsible for the reaction it is said to catalyze.

11. Your comment that resistance to 5'-nucleotidase is not a proof of structure is quite irrelevant to this paragraph. An assay was developed for an enzyme activity which made a 5'-deoxynucleotide insensitive to 5'-nucleotidase. We found that the only significant reaction, even with crude preparations, was the conversion of such nucleotides to the di- and triphosphates. This assay, therefore, served as a useful way to purify this enzyme(s) from extracts of E. coli.

12. The use of the term "polymerase", as stated in Paper I (page 3, line 1) "is for ease of reference in these reports" and is always contained within quotation marks. We have always been reluctant to name an enzyme until fuller knowledge of its activities was available. The Editors have not suggested another term, and for lack of a better one we choose to call this enzyme tentatively by this name.

13. The activity referred to as DNase B is measured by the release of acid-soluble fragments from DNA. It can be clearly distinguished from DNase A by its activity at high pH as described on page 21. These serologic experiments were carried out with the close cooperation of a distinguished immunologist, and we have had further consultation with him in the light of your criticism. Considerable work has been condensed into a few lines in order to establish the point that immunologic as well as fractionation procedures have indicated the existence of at least two discrete nucleases. In addition, the immunologic data provide evidence for the existence of two nucleases within the DNase B group. The importance of this information has been stressed in paragraph 5 above. It is our feeling that it would be difficult to go into any greater detail without making this section unmanageably long.

14. I object to the arbitrary nature of this comment. I will concede that the first paragraph can be omitted without harm as far as many biologically oriented readers of the Journal are concerned, but it is essential for a majority of the readers. However, if you disagree, I would be willing to omit the first paragraph.

15. You have missed the point of this argument. We are speaking only about deoxyuridine 5'-phosphate. We have said nothing about other "unnatural" pyrimidines or the presence or absence of kinases for them.

16. The designations of dATP, dCTP, and dGTP are certainly acceptable. Although I have now included the abbreviation of dTTP, I think it is very confusing.

17. References have been provided as you requested except for streptomycin, which was not used as a nucleic acid precipitant. It was used for empiric reasons to fractionate proteins.

18. I agree that Figure 2 of Paper II is unnecessary for people in this field of work. However, I doubt whether more than a few of the readers would find this figure superfluous. However, I am willing to concede this point if you insist.

In view of the two months' delay already suffered by these manuscripts, I would greatly appreciate a prompt decision.

Sincerely yours,

AK/McK

Arthur Kornberg.

Encls.